10593307

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA05/000124

International filing date: 02 February 2005 (02.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/634,604
Filing date: 10 December 2004 (10.12.2004)

Date of receipt at the International Bureau: 22 April 2005 (22.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

PCT/CA 20050000124

CA05/124

PA 1290180

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APPLICATION NUMBER: *60/634,604*
FILING DATE: *December 10, 2004*

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16698 U.S.PTO

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Express Mail Label No. _____

16800 U.S.PTO 60/634604 121004

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Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max):

METHOD AND APPARATUS FOR EXTRACTING CHARGED PARTICLES FROM A MEDIUM

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Application Data Sheet. See 37 CFR 1.76

☑ Specification *Number of Pages* 16

☑ Drawing(s) *Number of Sheets* 6

☐ CD(s), Number of CDs _____

☐ Other (specify) _____

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF FILING FEES AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

TOTAL FEE AMOUNT ($): $100.00

☑ Applicant claims small entity status. See 37 CFR 1.27.

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SIGNATURE _____ Date 9 DECEMBER 2004

TYPED or PRINTED NAME GAVIN N. MANNING REGISTRATION NO. 36,412 *(if appropriate)*

TELEPHONE (604) 669 3432 Docket Number: U008 0595

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METHOD AND APPARATUS FOR EXTRACTING CHARGED PARTICLES FROM A MEDIUM

Technical Field

[0001] This invention relates to methods and apparatus for extracting charged particles from a medium. The methods may be applied to extracting charged particles from electrophoresis substrates.

Background

[0002] Electrophoresis involves directing the movement of charged particles in a medium, such as a gel or liquid solution by applying an electric field across the medium. The electric field may be generated by applying a potential across electrodes that are placed in the medium. The movement of the particles in the medium is affected by the magnitude and direction of the electric field, the electrophoretic mobility of the particles and the mechanical properties of the medium. Through electrophoresis, particles that are distributed in a medium can be transported through the medium. Electrophoresis is commonly used to transport nucleic acids (such as DNA or RNA) through gel substrates. Since different species have different electrophoretic mobilities, electrophoresis may be used to separate different species from one another. Conventional electrophoresis techniques are largely limited in application to the linear separation of charged particles. Using conventional electrophoresis techniques, a direct current (DC) electric field or an alternating pulsed-field electrophoretic (PFGE) field is typically applied to a medium so that particles in the medium are transported toward an electrode.

[0003] One can isolate particles which have been separated from other particles by electrophoresis by cutting out the portion of the medium in which the particles have been carried by electrophoresis. The particles can be separated from the medium by using various purification techniques.

[0004] Laborious and expensive purification methods are typically used to prepare nucleic acid samples for biochemical analysis. Current techniques for the extraction of charged particles from fluids rely largely on centrifugation, filtration or magnetic separations, rather than electrophoresis. Such techniques often require expensive apparatus.

[0005] It is typically undesirable to use electrophoresis to concentrate nucleic acids. Electrochemical damage may occur to the nucleic acids upon interaction with an electrode.

[0006] In contrast with conventional electrophoresis techniques, the inventors have developed methods and apparatus which may be used to concentrate charged particles without requiring an electrode to be present in the zone where the particles become concentrated. Examples of these methods and apparatus are described in application No. 60/540,352 filed on 2 February 2004. Typical embodiments of these methods involve the application of a zero-time-averaged periodic driving force and a temporal alteration of the mobility or drag coefficient of the particles in the medium in which the particles are located. The periodic driving force can be in the form of an alternating electric field. By synchronizing the periodic driving force with the temporal alteration of the mobility or drag of the particles multi-dimensional electrophoresis can be achieved. In some cases the temporal alteration of the mobility or drag coefficient of the particles results from non-linear interactions between the particles and the medium. The principle on which this form of electrophoresis is based is hereinafter referred to as Synchronous Coefficient of Drag Alteration ("SCODA"). Electrophoresis based on SCODA may be used to concentrate polymers that are distributed through a uniform homogeneous medium toward a center region of the medium, which may be free from electrodes.

[0007] There remains a need for efficient ways to remove charged molecules or other particles from electrophoresis substrates and other media.

Summary of Invention

[0008] A method and apparatus for extracting charged particles from a medium is provided. A gel solution containing the particles to be extracted is placed next to a small amount of buffer in an extraction reservoir, creating a buffer-gel interface. Electrodes are provided on each side of the buffer-gel interface. By applying a pulsed voltage potential to the electrodes (wherein the time-averaged electric field is zero), zero-integrated-field electrophoresis (ZIFE) is applied to the buffer-gel interface to direct the particles in the gel into the extraction reservoir, where the particles are collected and concentrated.

[0009] This invention exploits differences in the mobility properties of particles in different media (such as a gel medium and a buffer medium) solutions. Some charged particles (such as molecules of DNA) exhibit an electrophoretic mobility in gel solution (such as agarose gel) that depends on the magnitude of the electric field applied. However, when those particles are in buffer or free solution, they have an electrophoretic mobility which is constant or at least has a much lower dependence on electric field strength. Application of ZIFE to a gel solution containing such particles will cause the particles to drift in the direction that yields the greater mobility. If the particles enter a region containing a buffer or free solution, they will stop drifting. Application of a zero time-averaged electric field will have no net drift effect on the particles in buffer solution.

[0010] A method according to one aspect of the invention comprises placing a buffer extraction reservoir next to a gel solution

containing the charged particles to be extracted; applying ZIFE to the buffer-gel interface to direct the particles into the extraction reservoir; and collecting and concentrating the particles in the extraction reservoir. A pipette or other device may then be used to suction the particles from the extraction reservoir.

[0011] In some embodiments of the invention, the apparatus comprises a gel boat holding a gel that contains the charged particles to be extracted. A capillary containing a small amount of buffer is inserted into the gel solution. A pipette or other device is provided in the capillary for suctioning the particles that have collected in the buffer. Electrodes are provided on each side of the buffer-gel interface for generating an electric field.

[0012] Further aspects of the invention and features of specific embodiments of the invention are described below.

Brief Description of Drawings

[0013] In drawings which illustrate non-limiting embodiments of the invention:

Figure 1 is a graphical illustration of an exemplary electric field pulse used in ZIFE;

Figure 2a is a cross-sectional elevation view of an extraction apparatus in accordance with a particular embodiment of the present invention, illustrating molecules of DNA in a solution prior to extraction;

Figure 2b is a cross-sectional elevation view of the apparatus of Figure 2a, illustrating molecules of DNA extracted from a solution and concentrated in a small amount of buffer;

Figure 2C is a plan view of an extraction apparatus similar to that shown in Figure 2a;

Figure 3 shows a glass capillary in an extraction experiment using the apparatus and method in accordance with a particular embodiment of the present invention;

Figure 4 is a graph illustrating the DNA fragment velocity during an experiment as a function of fragment length and cycle times; and,

Figure 5 shows a comparison between a DNA fragment mix and the fragment distribution of the same mix, after extraction.

Detailed Description

[0014] Throughout the following description, specific details are set forth in order to provide a more thorough understanding of the invention. However, the invention may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

[0015] This invention permits particles to be extracted from a medium. The invention may be applied to extracting charged biopolymers such as DNA, RNA and polypeptides from electrophoresis media, for example. Some embodiments of the invention use ZIFE (zero-integrated-field-electrophoresis) to move particles from a medium, such as an electrophoresis gel, into an adjacent fluid. ZIFE is a form of Alternating Current (AC) electrophoresis where, the polarity of an applied electric field reverses periodically and the time-averaged electric field is zero. The intensity of the electric field is greater in one polarity than in the other.

[0016] Figure 1 is a graphical illustration of an exemplary electric field pulse used in ZIFE. As shown in Figure 1, the pulse consists of an electric field E_1 applied in the positive, or "forward", direction for a time t_1, followed by an electric field E_2 applied in the negative, or "reverse", direction for a time t_2. If $E_2 = -E_1/r_e$ (where r_e is the field ratio), and $t_2 = t_1 r_e$ then the time-averaged electric field is zero. The time-averaged electric field is graphically represented by the shaded areas in Figure 1. The "positive" shaded areas (corresponding to E_1) cancel out the "negative" shaded areas (corresponding to E_2). Overall there is a zero net electric field. If the time-averaged electric field is exactly zero, then the ZIFE process is unbiased. However, if the time-averaged electric field slightly deviates from zero, then the ZIFE process is biased

[0017] The velocity v of a particle moving in a local electric field of amplitude E and having an electrophoretic mobility μ is given by:

$$v = \mu E \qquad \text{[Equation 1]}.$$

For linear systems, μ is constant. Particles having constant electrophoretic mobility have no net migration in a medium (*i.e.* their net velocity is zero) when ZIFE is applied to the medium. By contrast, in non-linear systems, particles have an electrophoretic mobility that is dependent on electric field amplitude. In such non-linear systems, there is a net migration of the particles in the direction that yields the greater mobility. In such a non-linear system, the particle velocity may be given by:

$$v = \mu(E)E \qquad \text{[Equation 2]}.$$

[0018] Suppose that charged particles in a medium have a field-dependent electrophoretic mobility of the form:

$$\mu(E) = \mu_0 + kE \qquad \text{[Equation 2A]}.$$

It can be seen that the mobility of these particles increases with the amplitude of the electric field E. The distance d travelled by the particles

under the influence of a constant electric field E is given by $d = vt$. If an electric field pulse of the form shown in Figure 1 is applied, the molecules will travel a greater distance during t_1 (while the pulse has the greater field amplitude) than the distance travelled during t_2. This can be shown by applying Equations 1 and 2A to the distance travelled by the particles. Hence, there is a net drift of particles in the "forward" direction, *i.e.* the direction in which the electric field of amplitude E_1 is applied. This net drift behaviour has been demonstrated by DNA molecules in agarose gels. In such gels, DNA molecules have an electrophoretic mobility of the form given by Equation 2. The field dependence of mobility arises from interactions between the DNA molecules and the gel. Therefore, ZIFE can be applied to DNA in an agarose gel to direct the DNA in a desired direction.

[0019] By contrast, application of ZIFE to DNA molecules in a buffer or free solution does not produce a net migration of DNA. This is because the mobility of the molecules in buffer solution is not field dependent. The differences in mobility properties of DNA in two media (e.g. a buffer and a gel) can be exploited to move particles from within one medium into another medium where the particles can be accumulated. This can be done by applying a ZIFE field across an interface between the two media.

[0020] Consider, for example, applying a ZIFE field across an interface between a gel in which there are DNA molecules and a buffer solution. Applying ZIFE to the molecules of DNA in the gel causes the molecules to migrate in the gel toward the gel-buffer interface. Once those molecules enter the buffer, the molecules will stop migrating. The ZIFE field may have a small bias in the direction which tends to move the molecules from the buffer toward the gel. This bias tends to prevent the molecules from diffusing too far away from the interface after they

enter the buffer. The bias may prevent the molecules from encountering the electrode used to create the ZIFE field. The bias is small enough that the particles in the gel continue to move toward the interface (i.e. the ZIFE velocity is not overcome by the net drift resulting from the bias).

[0021] A method and apparatus is provided wherein ZIFE is applied to a buffer-gel interface to extract DNA molecules from a gel and concentrate it in a small amount of buffer. This application of ZIFE is herein referred to as "Interface-ZIFE".

[0022] Apparatus according to one embodiment of the invention is shown in Figures 2a and 2b. Figure 2a shows molecules of DNA in a gel, prior to extraction, and Figure 2b shows molecules of DNA concentrated in a buffer, after extraction from the gel. An extraction apparatus **200** comprises a gel boat **20** (which may be shaped as a rectangular box) containing a gel **22**, such as agarose gel. Gel **22** fills a substantial volume of gel boat **20**. Preferably gel **22** is separated from each of electrodes **30B** by a buffer solution in a reservoir **24**. Reservoirs **24** are separated from one another so that the buffer does not provide short circuit paths between electrodes **30B**.

[0023] As shown in Figure 2a, prior to extraction, molecules of DNA **28A** are concentrated in a column in gel **22**. Molecules **28A** are typically not concentrated in such form when left in their natural state. Prior to being concentrated, molecules **28A** are typically distributed throughout gel **22**. To concentrate molecules **28A** into a column as shown in Figure 2a, SCODA-based electrophoresis as described in co-pending application No. 60/540,352 or another method may be applied to gel **22** so that molecules **28A** migrate to a region from where they are to be extracted. Concentration of molecules **28A** in a region of gel **22** is not

required prior to extraction. However, concentration is preferable to facilitate more efficient extraction of the molecules.

[0024] A capillary **25** containing a small amount of buffer solution is inserted into gel **22** so as to surround the molecules **28A** to be extracted. After inserting capillary **25** into gel **22**, the top portion of capillary **25** contains buffer solution, while the bottom portion of capillary **25** contains gel **22**. The buffer solution in capillary **25** provides an extraction reservoir **26** adjacent to gel **22**. Extraction reservoir **26** meets gel **22** at a buffer-gel boundary **21**. The arrangement of buffer and gel in capillary **25** forms a buffer-gel interface **31**. A pipette **29** is provided above capillary **25** to suction molecules **28A** after they have migrated into extraction reservoir **26**.

[0025] To provide the electric fields required for electrophoresis, an electrode **30A** is located near the tip of pipette **29**. Electrode **30A** is preferably located sufficiently far from the interface that the extracted molecules do not encounter electrode **30A** while the ZIFE field is being applied. A plurality of electrodes **30B** are located in buffer reservoir **24**. The electrodes may be made of platinum. More electrodes may be provided than those shown in Figures 2a, 2b. The tip of pipette **29** is filled with a small amount of buffer so as to provide conductivity between electrodes **30A** and **30B** when the pipette is inserted in capillary **25**. In one embodiment, electrodes **30B** are ganged to a fixed common potential (for example, electrodes **30B** may be grounded), while electrode **30A** is set to a different potential. A varying electric field can be applied across buffer-gel interface **31** by varying the potential of electrode **30A**.

[0026] To perform Interface-ZIFE, a zero time-averaged pulsed electric field is applied across buffer-gel interface **31**. The pulsed

electric field may be of the form that is shown in Figure 1. To cause molecules **28A** to migrate in the desired direction (*i.e.* toward extraction reservoir **26**), an electric field having an amplitude E_1 is applied in the direction toward extraction reservoir **26**, while an electric field having an amplitude E_2 is applied in the opposite direction. E_1 and E_2 are chosen such that the particles to be extracted have a greater mobility under the influence of E_1 than they do under the influence of E_2. For typical molecules and media $E_1 > E_2$. The polarity is selected so that the particles are driven toward interface **31** under the influence of E_1.

[0027] Application of Interface-ZIFE across buffer-gel interface **31** will cause molecules **28A** in gel **22** to drift toward extraction reservoir **26**. After some time, some of the molecules **28A** will cross buffer-gel boundary **21** and enter into the buffer in extraction reservoir **26**. Once these molecules reach extraction reservoir **26**, Interface-ZIFE has no net drift effect on the molecules and the molecules thus stop drifting. Eventually all (or most) of molecules **28A** will cross the buffer-gel boundary **21** and migrate into extraction reservoir **26**. Molecules **28A** become concentrated in the buffer adjacent the interface.

[0028] Figure 2b shows molecules **28B** (corresponding to molecules **28A** in Figure 2a) that have migrated from gel **22** into extraction reservoir **26**. Thus, Interface-ZIFE is used to collect and concentrate molecules **28B** in extraction reservoir **26**. Pipette **29** or another device can then suction molecules **28B** from extraction reservoir **26**, thereby completing the extraction process.

[0029] Figure 3 shows a glass capillary in an extraction experiment in which DNA mixed with a liquid gel was allowed to set within a capillary tube. Buffer was added to an upper portion of the capillary. The techniques described above were used to extract the DNA. An image of

the capillary was captured at various times (0 minutes, 60 minutes, 120 minutes) to show the effects of Interface-ZIFE applied to a buffer-gel interface. The buffer is a TAE (Tris-Acetate-EDTA) buffer and the gel is an agarose gel containing DNA. To perform this experiment, 100 μL of liquid 1% agarose gel, mixed with 5 μL λ DNA and 2.5 μg EtBr, was pipetted into the lower portion of a 2.5 mm inner diameter glass capillary and allowed to solidify. The upper portion of the capillary was filled with approximately 50 μL of 0.1X TAE buffer and a first platinum electrode was inserted into the buffer. The bottom of the capillary was then submerged in a shallow reservoir of 0.1X TAE buffer with a second platinum electrode. Interface-ZIFE was performed with these conditions: periodically, a voltage $V_1 = 200$ V was applied to the first electrode for a time $t_1 = 8$ s, then a voltage $V_2 = -100$ V was applied to the second electrode for $t_2 = 16$ s. The electric field was pulsed for 2 hours. The electrodes were separated by 5 cm. Over the course of the experiment, the upper half of the capillary remained filled with buffer and the DNA remained in a relatively small volume (approximately 20 μL). As shown by the images of the capillary, there is a progressive migration of DNA through a gel and concentration of the DNA in a small amount of buffer above the gel.

[0030] If extraction reservoir **26** is sufficiently small, then molecules **28B** that are concentrated in a region in extraction reservoir **26** will leave their concentrated region only by diffusion, which is slow over long distances. Convective mixing of molecules **28B** and extraction buffer **26** should be minimized to maintain molecules **28B** in their concentrated region. To minimize convective mixing, capillary **25** should preferably have a small diameter. Moreover, extraction buffer **26** and gel **22** are preferably at the same temperature.

[0031] In one embodiment, pipette **29** comprises a mechanized pipettor with built-in electrode **30A**. The mechanized pipettor aspirates buffer into a disposable pipette tip, then partially dispenses the buffer to cover the gel inside capillary **25** so that there is conductivity between electrodes **30A** and **30B**. Computer monitoring may be used to monitor the current between electrodes **30A** and **30B** during extraction, and detect such problems as bubbles or evaporation that may create an open circuit between the electrodes. After extraction is complete, the remaining buffer in the pipette tip is disposed of, and the pipette tip may return to capillary **25** to extract further samples of particles. Mechanized pipetting may reduce unnecessary pipette tip motion so that there is minimal mixing of the concentrated particles with the surrounding buffer. This minimizes the extraction volume and hence increases final concentration of the particles to be extracted.

[0032] In another embodiment, instead of inserting a capillary filled with buffer into the gel, the gel may be cast with a cavity. The cavity is filled with a buffer solution, and a pipette having an electrode is inserted into the buffer. The cavity functions similarly to the capillary in collecting the particles for extraction

[0033] Interface-ZIFE extraction of DNA mixtures from gels may be applied to selectively extract DNA fragments according to their size. If cycle times t_1 and t_2 for the electric field pulse are chosen to be sufficiently small, the relaxation or re-orientation time of the DNA molecules becomes significant and introduces a length-dependence in the migration velocity of the molecules. Figure 4 is a graph illustrating the DNA fragment velocity during an experiment as a function of fragment length and cycle times t_1 and t_2. In that experiment, DNA fragments of different lengths were linearly separated using standard DC electrophoresis in a 1% agarose gel (0.1X TAE). ZIFE was then applied

(in a direction perpendicular to that in which the DC electrophoresis was performed) to observe non-linear velocity of the fragments.

[0034] Figure 5 shows a comparison between a DNA fragment mix and the fragment distribution of the same mix, after Interface-ZIFE extraction. The mix was composed of 2 μL λ DNA (48kb, 500 ng/μL) and 4 μL 1 kb DNA ladder (0.5 - 10 kb, 500 ng/μL) and was run in 100 μL of 1% agarose gel applying the Interface-ZIFE extraction method described above. A pulsed electric field was applied, generated by a voltage $V_1 = 200V$ applied to the electrode in the pipette for a time of $t_1 =$ 25 ms, which alternated with a voltage $V_2 = -100V$ applied to the electrodes in the gel for a time of $t_2 = 50$ ms. The pulsed electric field was applied for 3 hours. This process extracted DNA into 0.1X TAE buffer which was mixed with loading dye and inserted into the well of a 1% agarose gel, along with a control from the original mix, for standard DC electrophoresis. The λ DNA band and short (less than 1 kb) fragments were not extracted from the gel. The size selection of Interface-ZIFE may be applied to longer fragments (100 - 200 kb) as well.

[0035] Parameters that can be varied to optimize extraction speed, extraction efficiency and DNA fragment length selectivity, include: magnitude of the electric pulsed field; frequency (cycle times) of the electric pulsed field; composition of the buffer in extraction reservoir **26**; composition of gel **22**; operating temperature; and the degree of concentration of molecules **28A**.

[0036] The methods and apparatus disclosed herein may be applied for extracting charged particles from a medium where the particles are concentrated in a particular region of the medium (such as DNA molecules concentrated in a column or pillar in gel). However, the

methods and apparatus are not limited to such application. They may also be employed to extract charged particles that are uniformly dispersed in the medium, located or concentrated in particular regions or bands, or otherwise distributed in the medium. Using the methods and apparatus disclosed herein, charged particles, and in particular biopolymers (for example, DNA, RNA and polypeptides), may be extracted from acrylamide, linear poly-acrylamid, POP (Perkin Elmer), agarose gels, entangled liquid solutions of polymers, viscous or dense solutions, solutions of polymers designed to bind specifically to the molecules whose motion is being directed, simple aqueous solutions, and the like. Interface-ZIFE used in conjunction with SCODA-based electrophoresis (for concentrating the DNA in a region) can be used to extract bacterial artificial chromosomes, plasmids and high molecular weight or genomic DNA.

[0037] As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations and modifications are possible in the practice of this invention without departing from the spirit or scope thereof, including but not limited to the following:

- The extraction of uncharged, or electrically neutral, molecules may be accomplished using the methods and apparatus disclosed herein if those molecules are carried by charged molecules. For example, neutral proteins that interact with charged micelles may be extracted electrophoretically through their interaction with the micelles.
- In place of unbiased ZIFE, biased ZIFE may be used to direct particles from the medium to the buffer. Biased ZIFE may facilitate selective separation of the particles according to their size.

[0038] It will be understood that this invention has many aspects including:

1. A method for collecting particles near an interface between first and second media, the method comprising:

providing particles in the first medium;

in alternation, for a first period applying a first field across the interface, the first field causing the particles to move toward the interface with a first velocity;

for a second period, applying a second field across the interface, the second field causing the particles to move away from the interface with a second velocity less than the first velocity;

wherein a time average of the first velocity over the first period is greater than a time average of the second velocity over the second period; allowing the particles to cross the interface into the second medium.

2. A method according to item 1 comprising allowing the particles to become concentrated in the second medium.

3. A method according to item 1 or item 2 wherein the particles comprise DNA.

Abstract of the Disclosure

A method and apparatus for extracting charged particles from a medium is provided, using zero-integrated-field electrophoresis (ZIFE) applied across a buffer-gel interface. A gel solution containing the particles to be extracted is placed next to a small amount of buffer in an extraction reservoir, creating a buffer-gel interface. Electrodes are provided on each side of the buffer-gel interface. By applying a pulsed voltage potential to the electrodes to generate a zero time-averaged electric field, ZIFE is performed to direct the particles in the gel into the extraction reservoir, where the particles are concentrated and collected. Molecules of genomic DNA, bacterial artificial chromosomes, and plasmids may be extracted using the method and apparatus described herein.

Figure 1

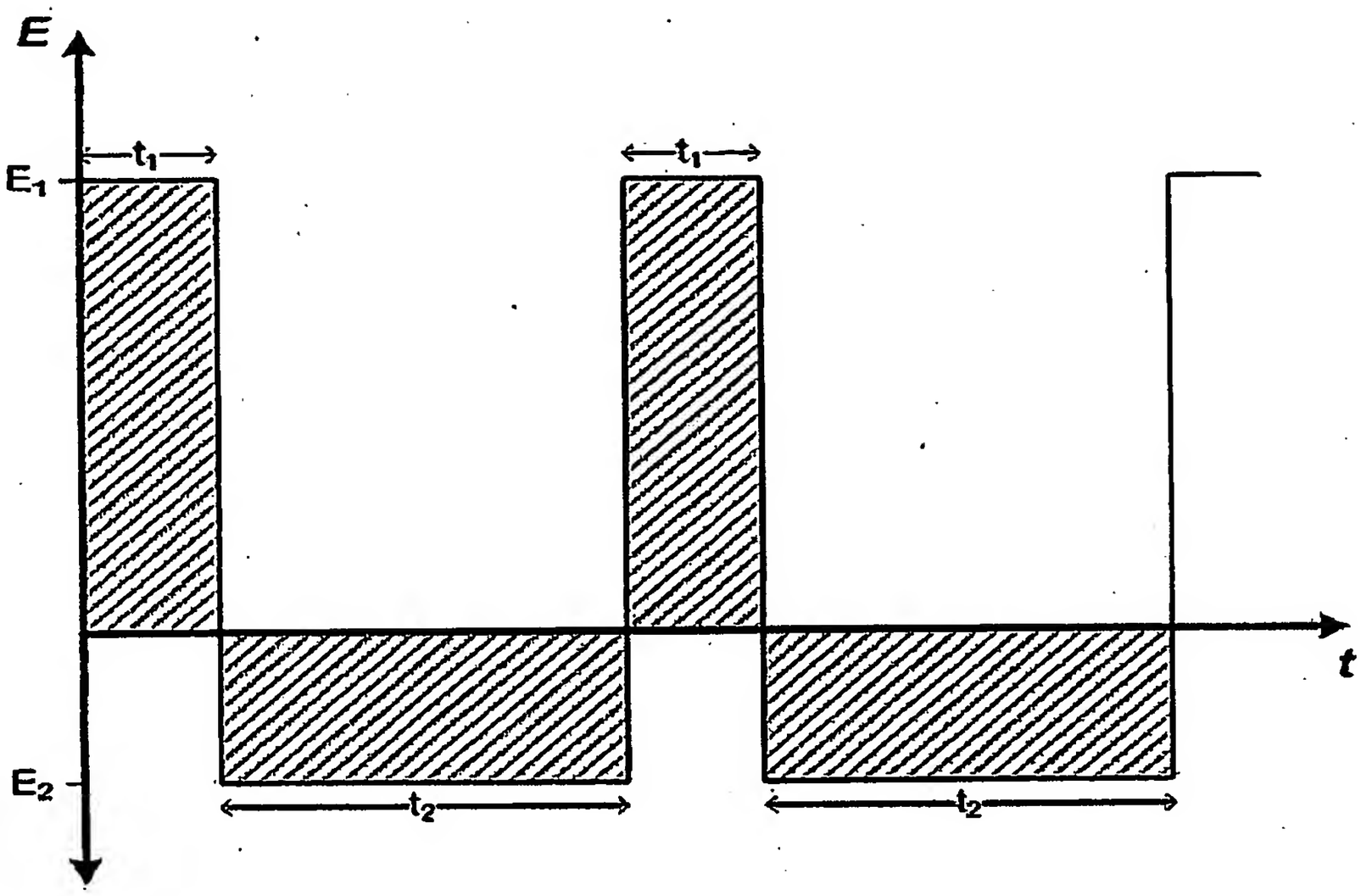

Figure 2a

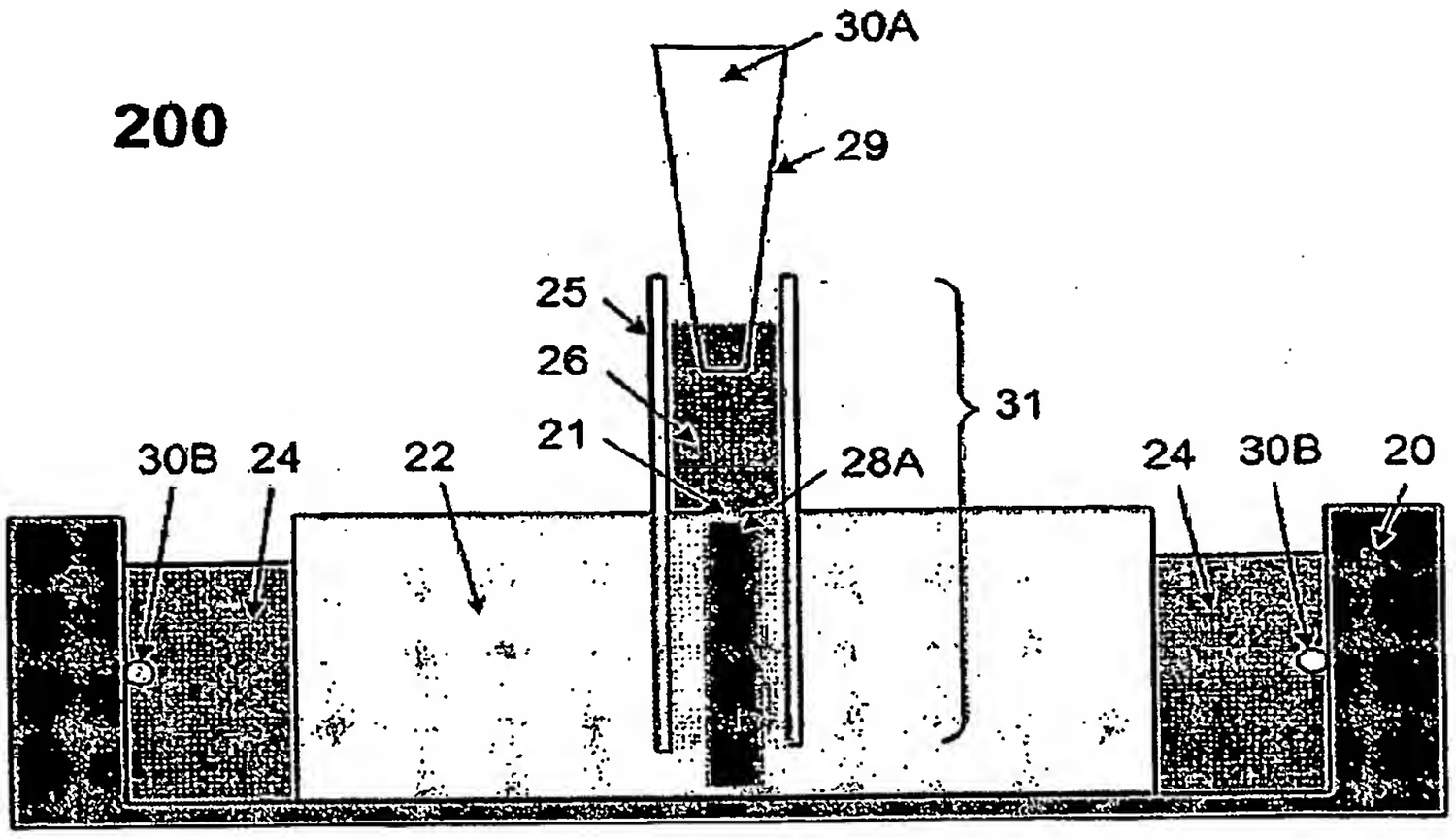

Figure 2b

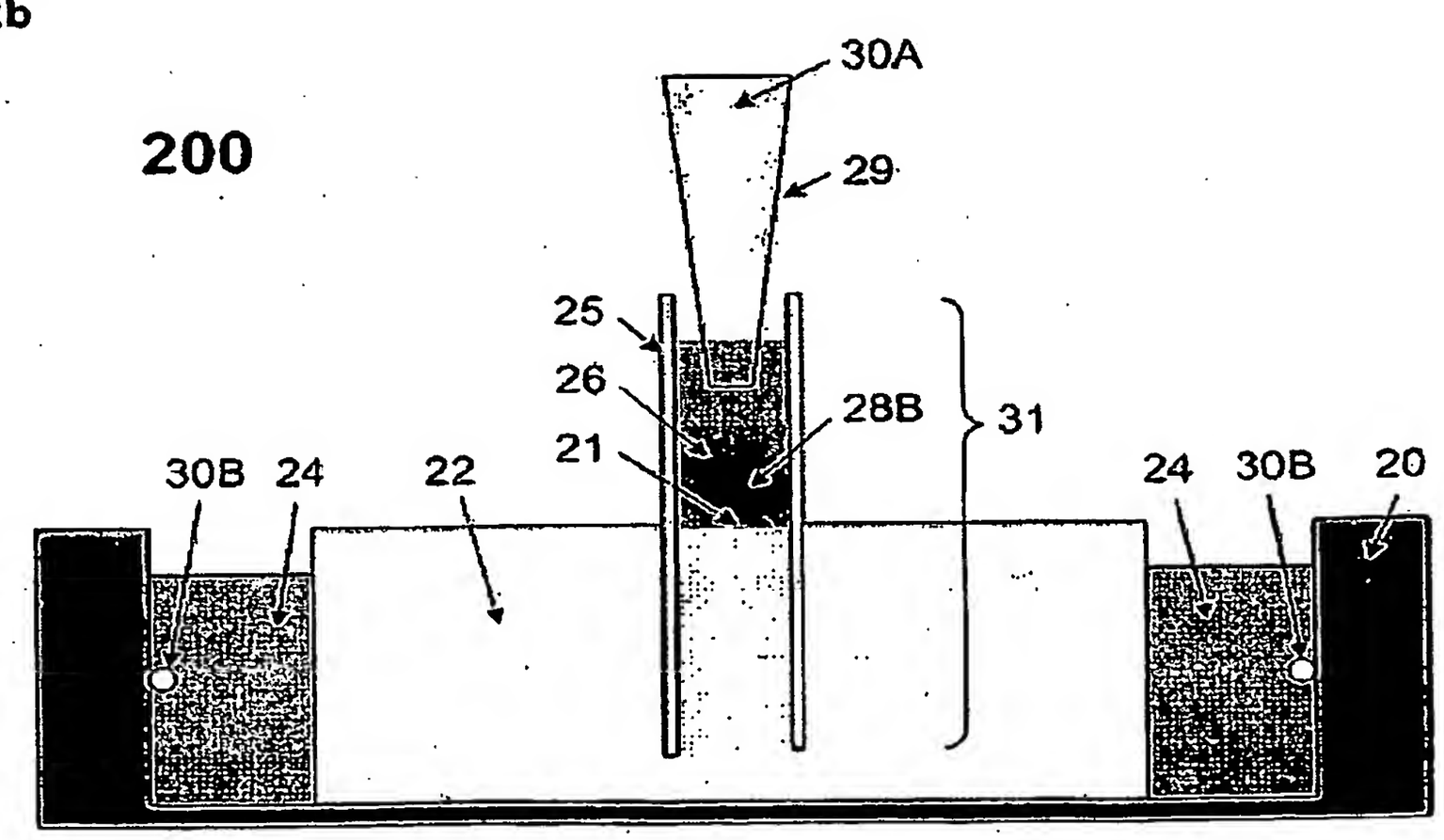

Figure 2c

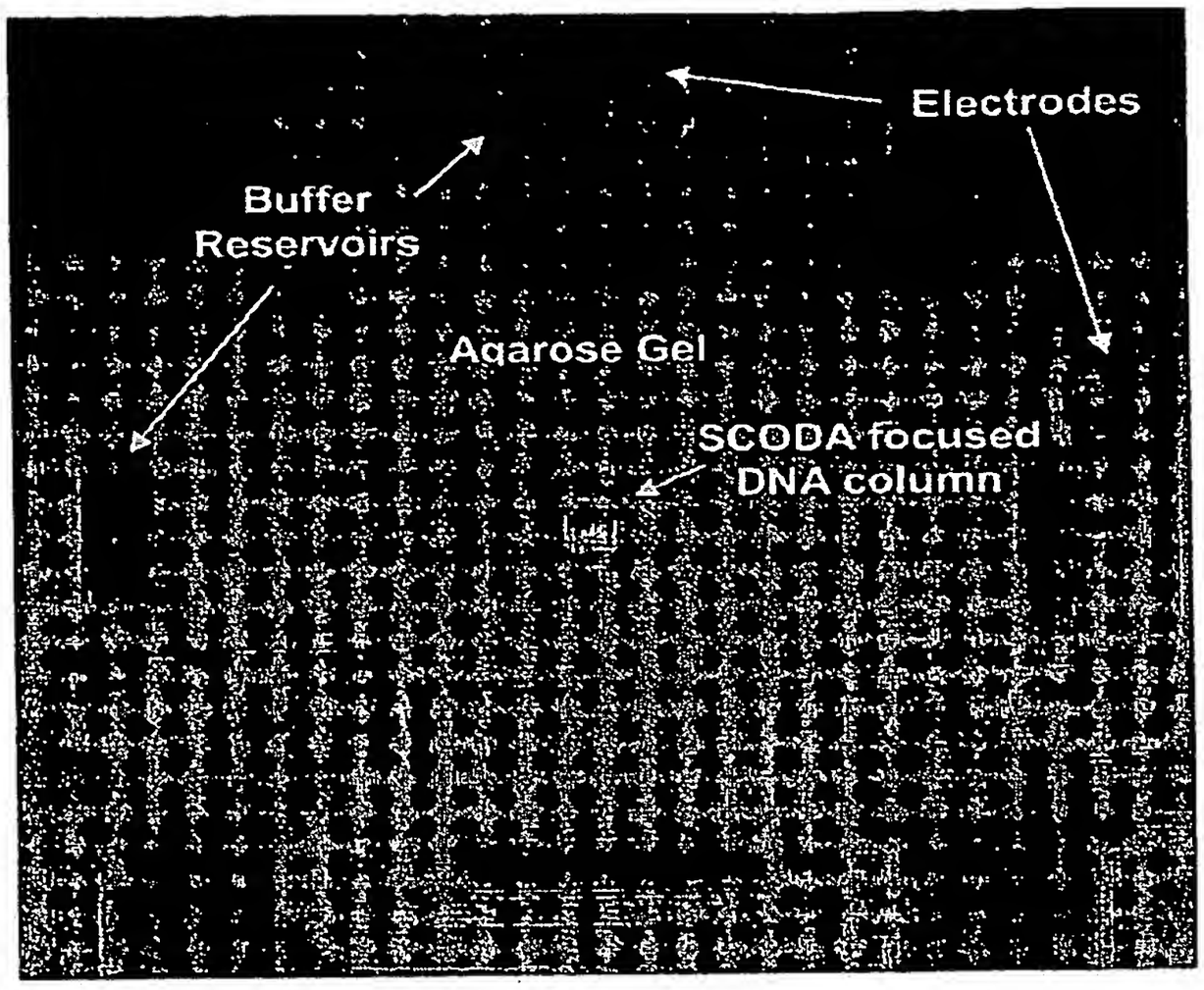

Figure 3

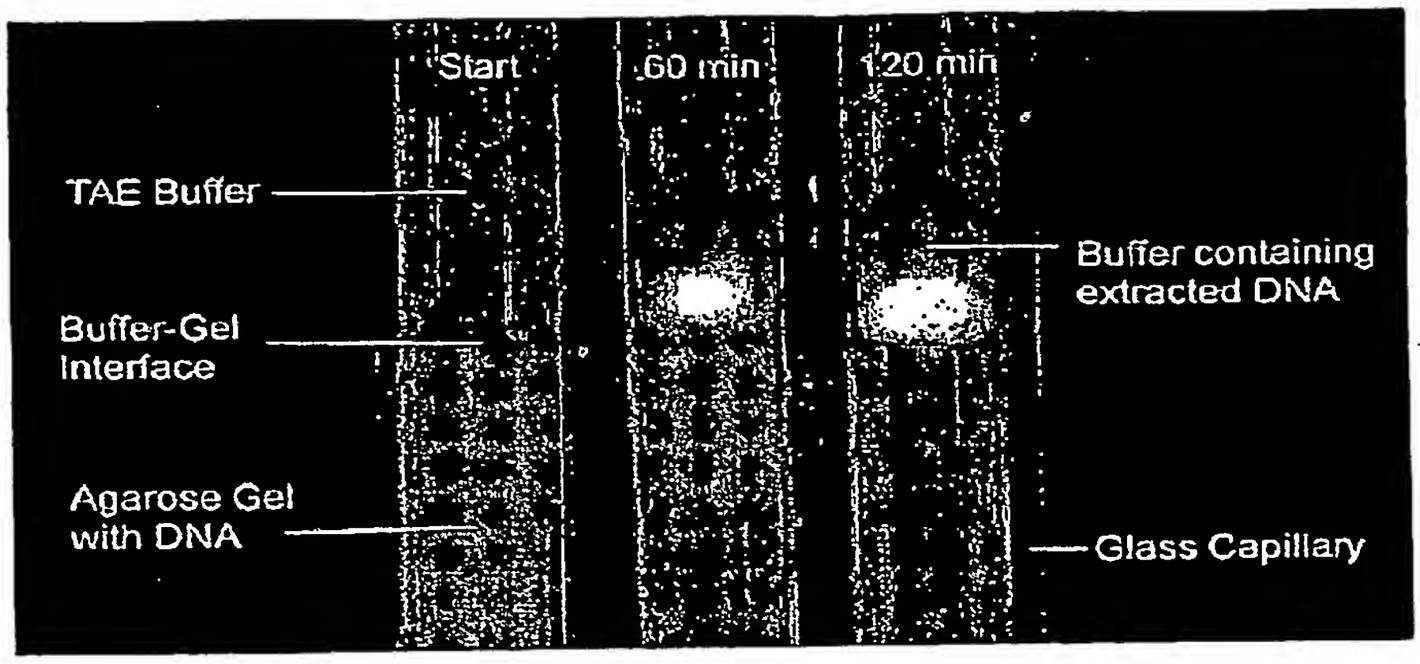

Figure 4

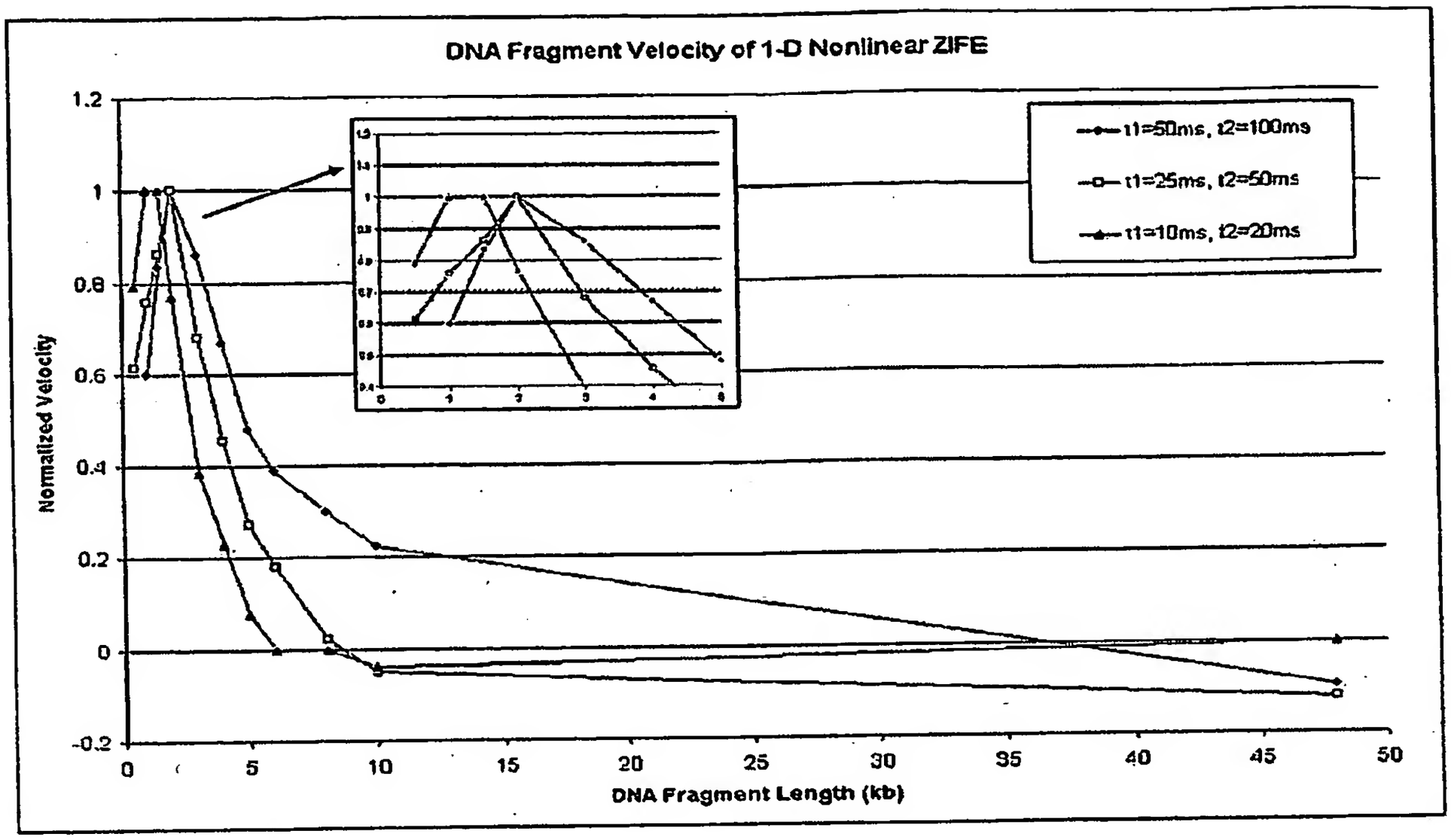

Figure 5